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14. ABSTRACT
It is clear that chemotherapeutic drugs produce cognitive deficits in humans. As it is not possible to measure neurogenesis in living humans, data must be obtained from experimental animals. So far, our data show that methotrexate reduces hippocampal neurogenesis. Thus, our animal model might be useful to determine which chemotherapeutic drugs would be likely to produce cognitive deficits in humans. The chemotherapeutic drug, methotrexate, impairs spatial working memory in our animal model, as reflected by decreased performance in the spontaneous task. The finding that cyclophosphamide affected neither cognitive function nor neurogenesis suggests not all chemotherapeutic drugs necessarily produce adverse cognitive effects in humans. If we identify drugs that reduce methotrexate-induced impairments in cognition and methotrexate-induced decreases in neurogenesis, we will be able to provide strong support for our hypothesis that chemotherapeutic agents produce cognitive impairment by disrupting hippocampal neurogenesis. As such, our animal model might be of utility to discover therapeutic strategies to prevent and/or treat the development of neurocognitive deficits in individuals undergoing cancer chemotherapy.

15. SUBJECT TERMS
Hippocampal neurogenesis, chemotherapeutic drugs, cognitive deficit, chemobrain, cancer

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Introduction

Post-chemotherapy cognitive impairment has been most often associated and studied in patients who have received adjuvant treatment for breast cancer. In the hippocampus, neural stem cells and progenitors proliferate and differentiate into neurons throughout adulthood. This phenomenon, called neurogenesis, is under the control of cell cycle regulators. Because some chemotherapeutic agents act by inhibiting cell cycle progression, our hypothesis is that chemotherapeutic agents produce cognitive impairment by disrupting hippocampal neurogenesis. The experiments in this Concept proposal will begin to develop and utilize an animal model to study the effects of chemotherapeutic drugs on cognitive function and hippocampal neurogenesis. The first specific aim will define the effects of these drugs on behavior. The second specific aim will determine whether they disrupt hippocampal neurogenesis. The third specific aim will ascertain if the behavioral deficits and inhibition of neurogenesis can be decreased or prevented by drugs that stimulate neurogenesis. Mice will be treated with saline, a single drug, or a combination of chemotherapeutic drugs, with and without drugs that stimulate neurogenesis. Neurological and behavioral function will be assessed, and hippocampal neurogenesis will be measured in brain sections by labeling cells with bromodeoxyuridine as well as by using other markers of neuronal proliferation. The results of these experiments could provide new and potentially important information on the fundamental mechanisms underlying post-chemotherapy cognitive impairment, and lead to the development of treatment strategies to treat and/or prevent this frequent and troubling problem in patients treated for breast cancer.

Body

Specific Aim 1: Define the effects of chemotherapeutic drugs on behavior.

Task 1. Dose-response study using single drugs (months 1-4)

Female mice are ordered at 5 weeks of age. At 7 weeks of age, the mice are injected i.p. with saline (0.9%), cyclophosphamide (10 mg/kg, 30 mg/kg or 100 mg/kg), methotrexate (3 mg/kg, 10 mg/kg or 30 mg/kg) or 5-fluorouracil (10 mg/kg, 30 mg/kg or 100 mg/kg). The mice are injected again one week and two weeks following the first injection. One week after the last injection the mice underwent behavioral testing according to the following schedule:

Day 1: Open Field, Neuroscreen
Day 2: Spontaneous Alternation
Day 3: Fear Conditioning (Acquisition)
Day 4: Fear Conditioning (Context)
Day 5: Fear Conditioning (Cue)

In order to determine whether drug treatment would produce behavioral effects, we started this task by administering the highest doses of each drug: cyclophosphamide (100 mg/kg); methotrexate (30 mg/kg); and 5-fluorouracil (100 mg/kg). We found unexpected toxicity after treatment with 5-fluorouracil; some subjects died after this dose, and additional mice has to be treated in order to obtain a sufficient number of subjects in this treatment group. Although the cyclophosphamide- and methotrexate-treated subjects were normal, the neuroscreen indicated that the 5-fluorouracil-treated mice showed neurological deficits. Compared to saline-treated controls, treatment with cyclophosphamide or methotrexate did not affect locomotor activity (Fig. 1) or rearing behavior (Fig. 2). In contrast, treatment with 5-fluorouracil reduced both locomotor activity (Fig. 1) and rearing behavior (Fig. 2). The results indicate that this dose of fluorouracil produced nonspecific toxic effects. Spontaneous alternation is a test of spatial working memory and thought to reflect hippocampal-dependent processes. Treatment with methotrexate produced deficits in spontaneous alternation (Fig. 3), but cyclophosphamide or 5-fluorouracil had no effect. None of the drug treatments affected fear conditioning.

These results show that treatment with methotrexate produced a specific deficit in performance of the spontaneous alternation task. This supports our hypothesis that an animal model can be developed and used to study cognitive deficits that occur as a consequence of treatment with chemotherapeutic drugs. At the present time, we are completing the testing of the two lower doses of each drug. In addition, we are testing more subjects in the fear conditioning procedure to see if we can detect an impaired context-specific response after modifying the testing parameters.

Figure 1

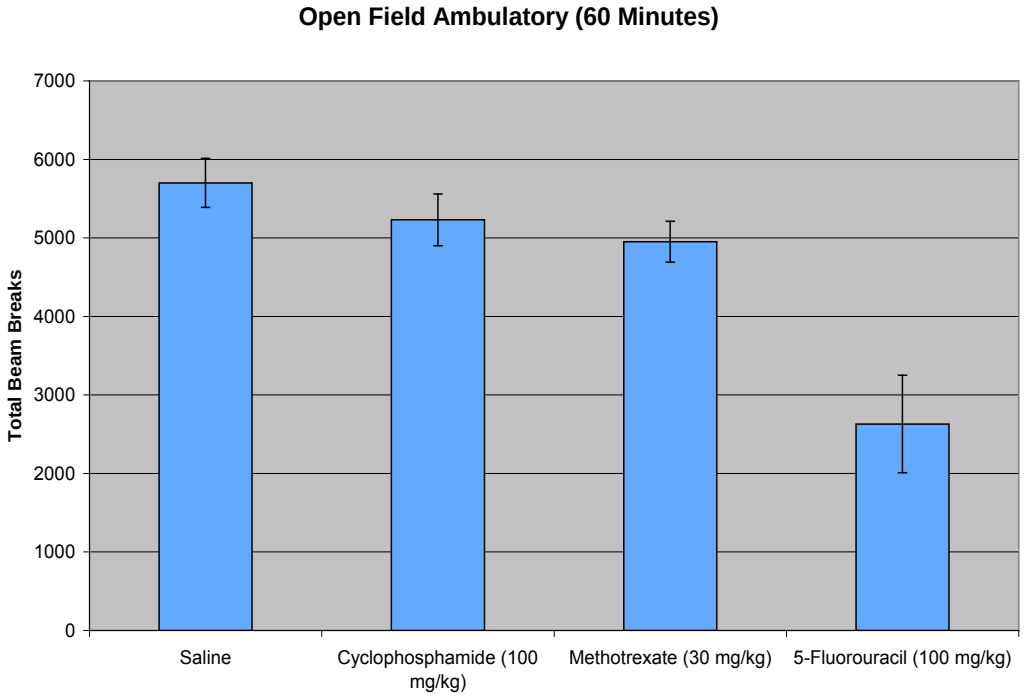


Figure 2

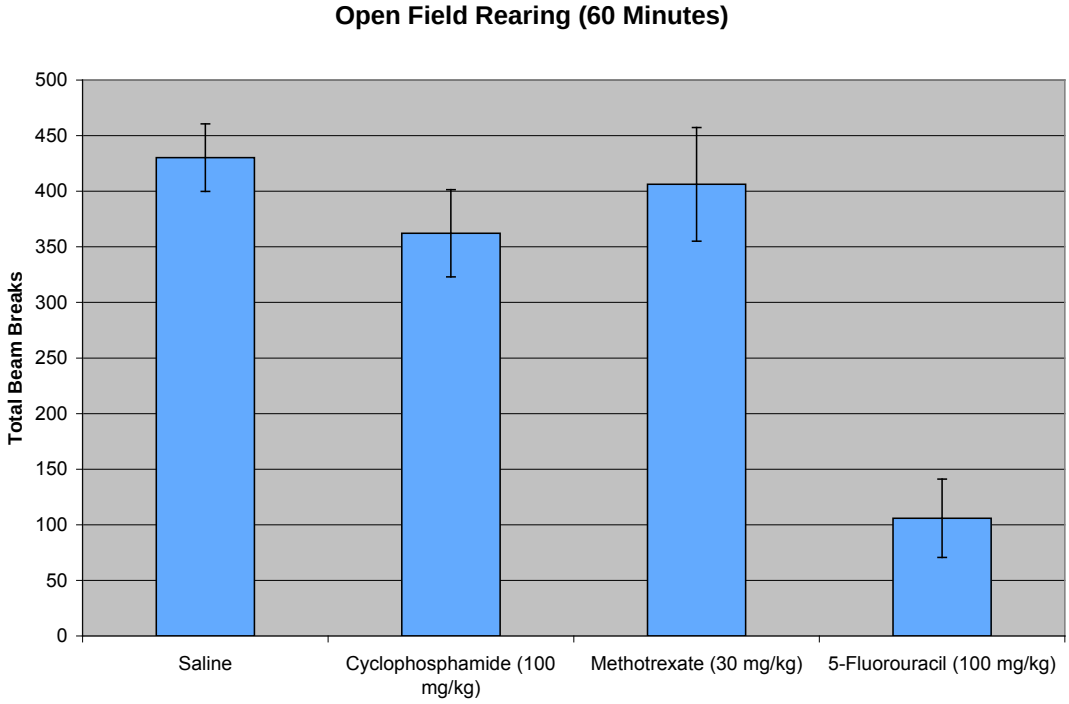
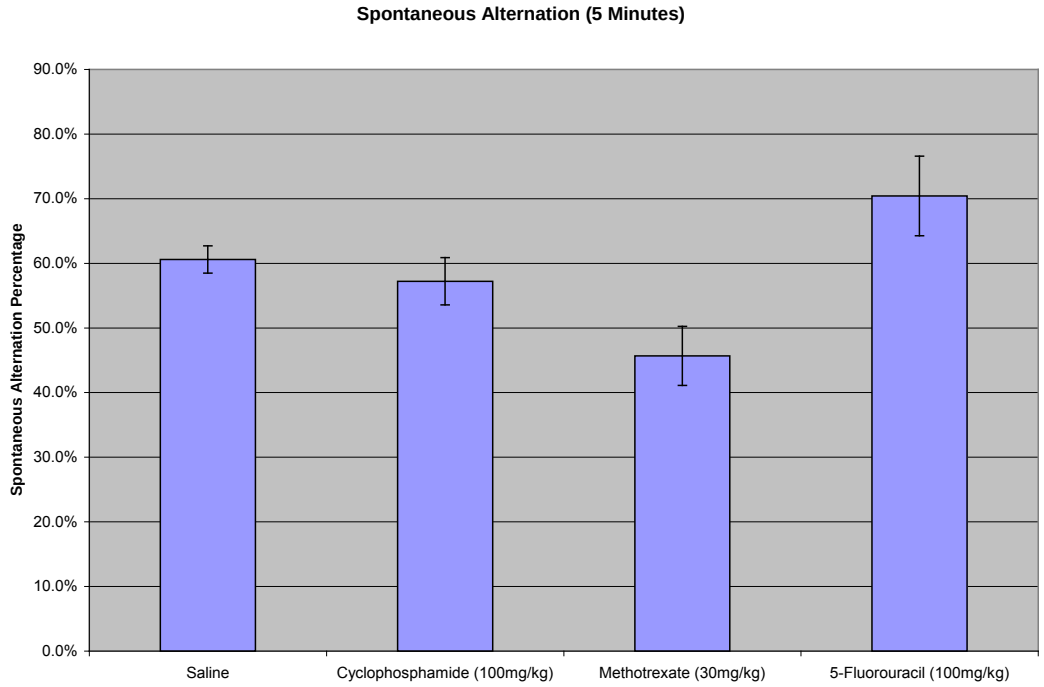


Figure 3



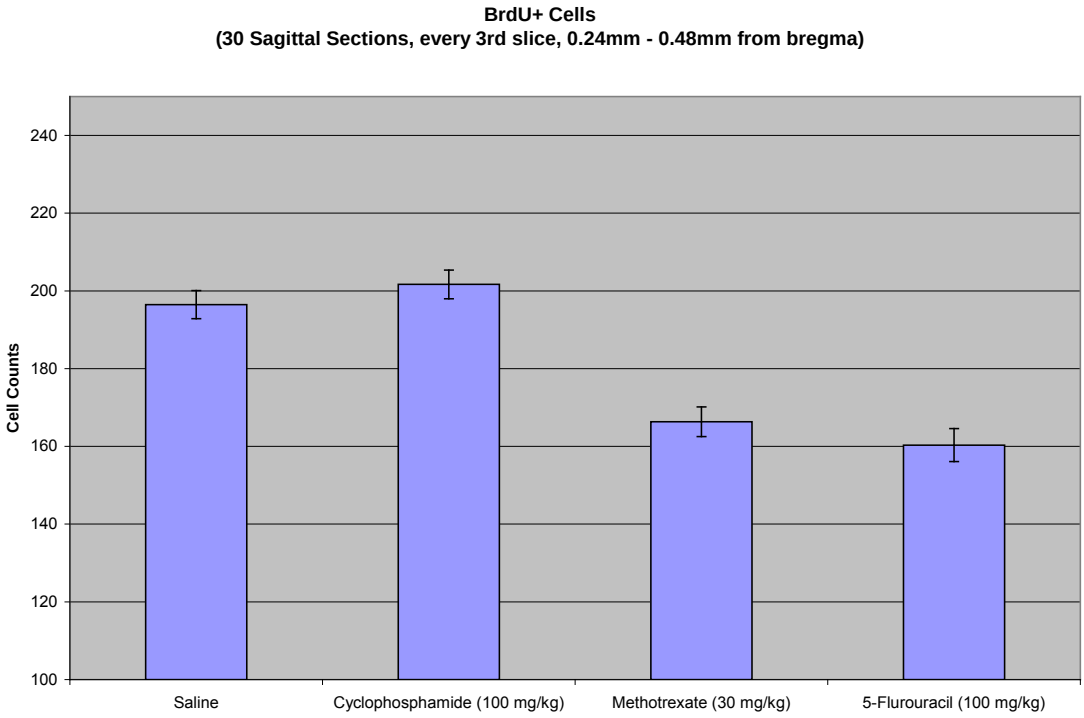
Task 2. Combination drug study (months 5-8)
This task could not be initiated prior to the completion of Task 1. We have been granted a no-cost time extension through 3/31/10, and Task 2 will be completed within this time frame.

Specific Aim 2: Determine effects of chemotherapeutic drugs on neurogenesis in the hippocampus.
Task 3. Dose-response study using single drugs (months 1-4)
Female mice are ordered at 5 weeks of age. At 7 weeks of age, the mice are injected i.p. with saline (0.9%), cyclophosphamide (10 mg/kg, 30 mg/kg or 100 mg/kg), methotrexate (3 mg/kg, 10 mg/kg or 30 mg/kg) or 5-fluorouracil (10 mg/kg, 30 mg/kg or 100 mg/kg). The mice are injected one week and two weeks following the first injection. Six days after the last injection the mice are treated with bromodeoxyuridine BrdU (50mg/kg/i.p.) every 2 hours for a total of 4 injections. Twenty-four hours after the first BrdU injection, the mice are sacrificed and the brains were stored in formalin prior to embedding in paraffin and sectioning.

In order to determine whether drug treatment would affect neurogenesis, we started this task by administering the highest doses of each drug: cyclophosphamide (100 mg/kg); methotrexate (30 mg/kg); and 5-fluorouracil (100 mg/kg). We found that BrdU labeling was markedly decreased in mice treated with methotrexate and 5-fluorouracil (Fig. 4). We have carried out TUNEL staining and found that there was no increase in apoptosis (programmed cell death) in these treatment groups. These findings indicate that treatment with methotrexate (30 mg/kg or 5-fluorouracil (100 mg/kg)) decreases neurogenesis. Because this dose of 5-fluorouracil caused lethality and nonspecific behavioral effects, we believe the effect of this drug on neurogenesis is due to nonspecific toxicity. We co-stained the tissue slides for NeuN and GFAP to show that the BrdU is labeling neurons (i.e., NeuN-stained cells) and not glia (i.e., GFAP-stained cells). We currently are analyzing these data. It is interesting to note the cyclophosphamide affected neither behavior nor neurogenesis, whereas methotrexate disrupted both performance on tests that measure cognitive function and hippocampal

neurogenesis. This finding supports our hypothesis that chemotherapeutic agents produce cognitive impairment by disrupting hippocampal neurogenesis. In carrying out this study, we found that it took much longer to section the tissue than originally anticipated. This was complicated further by limited access to a shared microtome. With practice, we now are able to process the tissue much faster, and we have identified another microtome that we can use for tissue slicing. At the present time, we are completing the testing of the two lower doses of each drug. All subjects have been treated and the tissue has been sectioned; we are finishing up the tissue staining and determination of BrdU labeling.

Figure 4



Task 4. Combination drug study (months 5-8)
This task could not be initiated prior to the completion of Task 3. We have been granted a no-cost time extension through 3/31/10, and Task 5 will be completed within this time frame.

Specific Aim 3: Test whether drugs that stimulate neurogenesis can prevent or decrease the behavioral deficits produced by chemotheraputic drugs.
Task 5. Effects of treatment on drug-induced behavioral deficits (months 9-12)
We have been granted a no-cost time extension through 3/31/10, and Task 5 will be completed within this time frame.

Task 6. Effects of treatment on neurogenesis (months 9-12)
We have been granted a no-cost time extension through 3/31/10, and Task 6 will be completed within this time frame.

Key Research Accomplishments

- Treatment with the chemotherapeutic drug methotrexate disrupted performance on the spontaneous alternation test, but did not produce nonspecific deficits in behavior. Spontaneous alternation is a test of spatial working memory and thought to reflect hippocampal-dependent processes. Therefore, the data suggest that treatment with methotrexate disrupted hippocampal function.

- Treatment with the chemotherapeutic drug methotrexate reduced hippocampal neurogenesis. The reduction in BrdU labeled was not due to increase apoptosis.
- These results support our hypothesis that chemotherapeutic agents produce cognitive impairment by disrupting hippocampal neurogenesis.
- The data support the development and utilization of this animal model to study the effects of chemotherapeutic drugs on cognitive function and hippocampal neurogenesis.

Reportable Outcomes

Pechnick, R.N., Reyes, K.C., Das, M, Lacayo, L.M., Farrokhi, C., Zonis, S. and Chesnokova V. Cognitive impairment and decreased hippocampal neurogenesis after treatment with chemotherapeutic drugs. To be presented at the Experimental Biology meeting, Anaheim, California, 2010. (see Appendix)

Conclusion

The results so far are very encouraging. It is clear that chemotherapeutic drugs produce cognitive deficits in humans. Currently, it is not possible to measure neurogenesis in living humans while they are alive, so data must be obtained from experimental animals. Our data show that methotrexate reduces hippocampal neurogenesis. Thus, our animal model might be useful to determine which chemotherapeutic drugs would be likely to produce cognitive deficits in humans. Our results show that the chemotherapeutic drug methotrexate impairs spatial working memory in our animal model, as reflected by decreased performance in the spontaneous task. The finding that cyclophosphamide affected neither cognitive function nor neurogenesis suggests not all chemotherapeutic drugs necessarily produce adverse cognitive effects in humans. The most important experiments in this project currently are underway. If we identify drugs that reduce methotrexate-induced impairments in cognition and methotrexate-induced decreases in neurogenesis, we will be able to provide strong support for our hypothesis that chemotherapeutic agents produce cognitive impairment by disrupting hippocampal neurogenesis. As such, our animal model might be of utility to discover therapeutic strategies to prevent and/or treat the development of neurocognitive deficits in individuals undergoing cancer chemotherapy.

Appendix

Please see attached abstract

Supporting Data

None

Cognitive impairment and decreased hippocampal neurogenesis after treatment with chemotherapeutic drugs

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Post-chemotherapy cognitive impairment has long been recognized in cancer survivors; however, at the present time the cause(s) is not known. The objective of the present study was to determine the effects of chemotherapeutic drugs on cognitive function and hippocampal neurogenesis in the mouse. Adult female mice were injected once a week for 3 weeks with methotrexate (30 mg/kg/i.p.), cyclophosphamide (100 mg/kg/i.p.) or saline (0.9%/i.p.). One week after the last injection they underwent behavioral testing. Another cohort of mice did not undergo behavioral testing, but were treated with bromodeoxyuridine (BrdU; 50 mg/kg/i.p.; every 2 hr for a total of 4 injections) and sacrificed 24 hr later. There were no significant differences in locomotor activity among the treatment groups; however, spontaneous alternation was impaired in the methotrexate-treated subjects. BrdU incorporation was not affected in the cyclophosphamide-treated subjects, but was markedly reduced in the dentate gyrus of the hippocampus after treatment with methotrexate. These results suggest that post-chemotherapy cognitive impairment might be linked to drug-induced decreases in hippocampal neurogenesis. Supported by Department of Defense Breast Cancer Research Program grant number BC075629.